

SILICA-SCALED CHRYSOPHYTES IN BULGARIA

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ABSTRACT — During an examination by transmission electron microscopy of samples from 21 freshwater localities (lakes, swamps, ponds and streams) in Bulgaria, a total of 13 species of silica-scaled chrysophytes (Chrysophyceae and Synurophyceae) were identified. Only three of these species had previously been recorded from Bulgaria, and only one of these was studied by electron microscopy. Eight of the species are cosmopolitan in their distribution, three are restricted to northern temperate regions, and two have a bipolar temperate distribution. Almost all the species have been reported from adjacent countries.

RÉSUMÉ — Treize espèces de chrysophytes à écailles silicieuses (Chrysophyceae et Synurophyceae) ont été identifiées par examen, en microscopie électronique à transmission, d'échantillons de 21 localités d'eau douce (lacs, étangs, tourbières et rivières), en Bulgarie. Trois de ces espèces étaient déjà signalées en Bulgarie, mais une seule avait fait l'objet d'une étude en microscopie électronique. Huit des espèces sont cosmopolites, les cinq autres sont limitées aux régions tempérées, dont deux avec une distribution tempérée bipolaire. Presque toutes ces espèces sont connues dans des pays limitrophes.

KEY-WORDS: Bulgaria, Chrysophyceae, distribution, freshwater algae, Heterokonta, new record, Stramenopiles, Synurophyceae, transmission electron microscopy.

INTRODUCTION

The flora of silica-scaled chrysophytes (belonging to the classes Chrysophyceae and Synurophyceae) in Bulgaria is very little known (Kristiansen *et al.*, in press). In contrast, there are several more in-depth investigations from the adjacent countries of Romania (e.g. the works by Péterfi, 1966; Péterfi & Momeu, 1976; Momeu & Péterfi, 1987) and Greece (Kristiansen, 1980, 1983), and also from Hungary (Kristiansen & Padisák, 1992; Kiss & Kristiansen, 1994).

In Bulgaria, some early finds of species of *Mallomonas* and *Synura* have been reported by Valkanov (1928), with light microscopy, but such observations are no longer considered reliable. These and other LM documented finds of chrysophytes have been listed and evaluated in the survey by Kristiansen *et al.*, in press. Electron microscopy of the

silica scales is indispensable for species identification within this group of organisms (see e.g. Kristiansen, 1996), but the only EM study as yet on Bulgarian material is the description of a bloom of *Mallomonas acaroides* Perty from a pond in the Rila Mountains (Kristiansen, 1971).

MATERIALS AND METHODS

The samples were collected from the water surface in 1 l glass or plastic bottles and fixed with 2-4 % formaldehyde. Electron microscopy of the silica scales was done in the Department of Phycology, University of Copenhagen. Droplets of sedimented material from the samples were dried onto formvar/carbon coated grids, washed in distilled water and after drying the grids were examined in a JEOL JEM 100SX electron microscope.

The 33 samples were taken from 21 localities (Fig. 1) that included some lowland temperate lakes (Loc. 1, 2), lowland as well as mountain swamps (Loc. 3, 4, 5, 6, 7), mountain stream and peatbogs (Loc. 8, 9, 10), high mountain lakes (Loc. 11, 12, 13, 14, 15, 16, 17, 18, 19, 20), and a high mountain pool (Loc. 21). The localities are located at about 42-44° N.Lat. Localities are listed in Table 1.



Fig. 1. Map of Bulgaria showing the location of the investigated localities.

Table 1. List of sampled localities.

Locality 1. Lake "Beloslavsko ezero" — located west to the town of Varna at the Black Sea Coast, area of 400 ha, maximum depth 3.5 m, average depth 2.3 m, volume $9 \cdot 10^6 \text{ m}^3$, previously freshwater, recently changed to brackish water (salinity $> 5\text{‰}$), surrounded by reeds. Sampled on 23.05.92 by Dr Delov from Sofia University (SU).

Locality 2. Srebarna Lake — located at the right bank of the Danube River, total area of 2.4 km^2 and open water area of 0.6 km^2 , maximum depth at the sampling time 1.2 m, volume $3.7 \cdot 10^5 \text{ m}^3$, pH varying between 7 and 8.5, freshwater, highly eutrophic holo-polymictic temperate lake surrounded by reeds. Sampled on 20.05.92, 6.06.92, 22.04.93, 6.07.93, 10.12.94 by Dr M. Stoyneva (SU), N. Michov, Dr M. Marinov, and Dr T. Michev from Bulgarian Academy of Sciences (BASc).

Locality 3. Swamp "Blato Tarlitza" — located at the right bank of the Danube River and adjacent to the Srebarna Lake, maximum depth at the sampling time 0.5 m, freshwater, highly eutrophic holo-polymictic temperate swamp. Sampled on 27.04.94 by Dr M. Stoyneva, N. Michov and Dr T. Michev.

Locality 4. Swamp (A) in the South Park of Sofia town — located in the central part of Sofia (about 560 m above sea level), maximum depth at the sampling time 0.8 m, freshwater holo-polymictic temperate swamp overgrown by hydrophytes. Sampled on 15.04.88 by Dr M. Stoyneva.

Locality 5. Swamp (B) in the South Park of Sofia town — located in the central part of Sofia (about 560 m above sea level), maximum depth at the sampling time 0.5 m, freshwater holo-polymictic temperate swamp surrounded by rushes. Sampled on 21.06.89 by Dr M. Stoyneva.

Locality 6. Swamp "Dolno Bojansko blato" — located in the south-western part of Sofia town at the foot-hills of Vitosha Mountain (about 610 m above sea level), maximum depth at the sampling time 0.4 m, freshwater holo-polymictic swamp with small open water areas among the rush-bed. Sampled 16.07.94 by Dr M. Stoyneva.

Locality 7. Swamp "Gorno Bojansko blato" — located in the south-western part of Sofia town at the foot-hills of Vitosha Mountain (about 620 m above sea level), maximum depth at the sampling time 1.2 m, freshwater holo-polymictic swamp with a rush-bed at its northern and *Sphagnum* at its southern part. Sampled on 16.07.94 by Dr M. Stoyneva.

Locality 8. Stream "Suhodolska reka" located in the south-western direction towards the town of Sofia, at about 580 m above sea level. Sampled on 12.06.92 by Dr M. Stoyneva, Dr St. Kovachev (SU), Dr T. Michev and Dr V. Velez (BASc).

Locality 9. Peat-bog at Vitosha Mountain — a small bog overgrown by *Sphagnum*, located at about 1000 m above sea level. Sampled on 18.07.94 by Dr M. Stoyneva.

Locality 10. Peat-bog in the Rila Mountains — a small bog located near the top of Maljovitz at about 2350 m above sea level. Sampled on 27.07.94 by Dr M. Stoyneva and Dr T. Michev.

Locality 11. Lake "Dolno Kamilsko ezero" — located in the Rila Mountains at about 2300 m above sea level. Sampled on 28.07.94 by Dr M. Stoyneva and Dr T. Michev.

Locality 12. Lake "Gorno Kamilsko ezero" — located in the Rila Mountains at about 2350 m above sea level by Dr M. Stoyneva and Dr T. Michev.

Locality 13. Lake "Strashnoto ezero" — located in the Rila Mountains at 2408 m above sea level, water surface area 0.14 ha, maximum depth 2.0 m, pH = 7.0. Sampled on 28.07.94 by Dr M. Stoyneva and Dr T. Michev.

Locality 14. Lake "Suhoto ezero" — located in the eastern part of the Rila Mountains at about 2475 m above sea level, water surface area 0.9 ha. Sampled on 19.10.94 by Dr Sp. Tonkov (SU) and Dr D. Dimitrov (SU). Locality 15. Lake 1 from the group "Urdini ezero" — located in the western part of the Rila Mountains at 2375 m above sea level, maximum depth 4.7 m, average depth 1.9 m, water surface area 0.9 ha, pH = 6.6. Sampled on 18.10.69 by Dr St. Draganov (SU).

Locality 16. Lake 2 from the group "Urdini ezero" — located in the western part of the Rila Mountains at 2278 m above sea level, maximum depth 6.6 m, average depth 3.6 m, water surface area 2.5 ha, volume $8.95 \cdot 10^4 \text{ m}^3$, pH = 6.8. Sampled on 18.10.69 by Dr St. Draganov.

Locality 17. Lake 3 from the group "Urdini ezera" — located in the north-western part of the Rila Mountains at 2339 m above sea level, maximum depth 4.7 m, average depth 2.5 m, water surface area 2.3 ha, volume $5.95 \cdot 10^6 \text{ m}^3$, pH = 7.7. Sampled on 18.10.69 by Dr St. Draganov.

Locality 18. Lake "Okoto na Todora" — located in the northern part of the Pirin Mountains at 2062 m above sea level, water surface area 0.3 ha, maximum depth 5.5 m, average depth 2.5 m, volume $6.6 \cdot 10^3 \text{ m}^3$. Sampled on 26.10.89 by Dr M. Stoyneva and Dr D. Lazarov.

Locality 19. Lake "Ribno ezero" — located in the northern part of the Pirin Mountains at 2190 m above sea level, water surface area 6 ha, maximum depth 12 m. Sampled on 30.06.92 by Dr V. Koicheva.

Locality 20. Lake "Banderishko ezero" — located in the northern part of the Pirin Mountains at 2312 m above sea level, water surface area 0.4 ha, average depth about 1 m. Sampled on 26.10.89 by Dr M. Stoyneva and Dr D. Lazarov.

Locality 21. A pool near the tourist house "Vichren" — in the northern part of the Pirin Mountains at about 2000 m above sea level, with mass development of *Chaetophora elegans* (Roth) C. Ag. Sampled on 26.10.89 by Dr M. Stoyneva and Dr D. Lazarov.

RESULTS AND DISCUSSION

A total of 13 species were found, including four belonging to the Chrysophyceae and nine to the Synurophyceae. They have been listed, together with the localities, in Table 2, and electron micrographs of their silica structures are given as Figs 2-19. Some of the scales appeared rather corroded, affecting the quality of several of the pictures. In Fig. 12, e.g., the secondary layer of the scale has almost disappeared, but it can be better seen in the original micrographs.

Table 2. Chrysophyte taxa and their occurrence in the investigated localities

<i>Spiniferomonas abei</i> Takahashi: Loc. 12
<i>Spiniferomonas trioralis</i> Takahashi: Loc. 2, 19
<i>Paraphysomonas takahashii</i> Cronberg & Kristiansen: Loc. 2
<i>Paraphysomonas vestita</i> (Stokes) De Saedeleer: Loc. 2 (four samples)
<i>Mallomonas matvienkoae</i> (Matvienko) Asmund & Kristiansen var. <i>matvienkoae</i> : Loc. 12
<i>Mallomonas papillosa</i> Harris & Bradley var. <i>papillosa</i> : Loc. 15, 20, 21
<i>Mallomonas caudata</i> Ivanov emend. Krieger: Loc. 5, 8, 19
<i>Mallomonas actinoloma</i> Takahashi var. <i>maramuresensis</i> Péterfi & Momeu: Loc. 2, 12
<i>Mallomonas alpina</i> Ruttner in Pascher: Loc. 19
<i>Mallomonas tonsurata</i> Teiling: Loc. 5, 8, 19
<i>Mallomonas acaroides</i> Perty emend. Ivanov var. <i>acaroides</i> : Loc. 5, 8, 15, 17, 18, 19, 20, 21
<i>Mallomonas pumilio</i> Harris & Bradley var. <i>munda</i> Asmund: Loc. 20
<i>Synura petersenii</i> Korshikov: Loc. 5, 8, 19, 22

The Chrysophyceae were represented by the genera *Paraphysomonas* and *Spiniferomonas*. *Spiniferomonas trioralis* (Fig. 3) is a widely recorded cosmopolitan species. It is recorded from neighbouring Greece (Kristiansen, 1980), and also from Hungary (Kristiansen & Padišák, 1992). *Spiniferomonas abei* (Fig. 2) has scattered records from temperate and arctic regions in the northern and southern hemisphere. In Europe it has hitherto been recorded from Denmark (Kristiansen, 1978) and Norway (Skogstad, 1986). The find in Bulgaria thus represents its southernmost reported European occurrence. *Paraphysomonas vestita* (Fig. 5) is one of the world's most widely distributed chrysophytes, found in all parts of the world. It has previously both been recorded from Greece (Kristiansen, 1980) and Hungary (Hajdu, 1975; Kristiansen & Padišák, 1992). *Paraphysomonas takahashii* (Fig. 4) has a much more scattered occurrence. Since its description from Sweden (Cronberg & Kristiansen, 1980) it has been found in several localities in northern temperate regions, in Asia, America and Europe. The European finds further include Iceland (Kristiansen, 1995), Denmark (Thomsen *et al.*, 1981), and Finland (Hällfors & Hällfors, 1988). Thus, it has never been recorded so far south as in Bulgaria before.

The Synurophyceae were represented by the two genera *Mallomonas* and *Synura*, with eight and one species, respectively. *Mallomonas matvienkoae* f. *matvienkoae* (Fig. 6) is a cosmopolitan taxon, occurring mainly in temperate and subtropical regions. It has also been recorded from Romania (Asmund & Kristiansen, 1986; Péterfi & Momeu, 1977), whereas another variety, var. *grandis* Dürrschmidt & Cronberg, has its main occurrence in the tropics (Cronberg, 1989; Wujek & Saha, 1996). *Mallomonas papillosa* var. *papillosa* (Fig. 10) is a widely distributed cosmopolitan taxon, also found in many European countries. It was recorded from Romania by Péterfi & Momeu (1976). *Mallomonas caudata* (Figs 8-9) has previously been recorded from the Bulgarian Danube section by LM (Szemes, 1967) and it is one of the few species which can be reliably identified in this way. It is an almost cosmopolitan species, and it has been recorded from the adjacent countries of Romania and Greece (Asmund & Kristiansen, 1986). *Mallomonas actinoloma* var. *maramuresensis* (Fig. 7) was described by Péterfi & Momeu (1976) from the Maramures Mountains in Romania. Later it has been shown to have a wide but scattered northern distribution, in Northern Europe and Japan (see Asmund & Kristiansen, 1986), but it has not been recorded from adjacent countries other than Romania. *Mallomonas alpina* (Fig. 11) is a common cosmopolitan species. It has also been found in the adjacent countries Romania and Greece, and also in Hungary (Asmund & Kristiansen, 1986; Kristiansen & Padišák, 1992). *Mallomonas tonsurata* (Figs 12-13) is one of the world's most widely distributed species. It has also been found in the neighbouring Greece and in Hungary (Kristiansen, 1980; Kristiansen & Padišák, 1992), but has apparently never been recorded from Romania. The species has previously been identified by LM on dried material collected in Bulgaria by Valchanova (1995) and by Stoyneva & Valchanova (1997). *Mallomonas acaroides* (Figs 14-17) was found in several localities. Specimens found in localities 5, 18 and 19 had scales with a very weakly developed ornamentation, whereas the scales in the other samples had a well developed reticulum on the shield. Most often both serrated bristles and helmet bristles are present, but in some localities only serrated bristles were found, in other localities only helmet bristles (Loc. 15, 16, 18, 21). The previous find of a population of this species in Bulgaria examined by EM showed all bristles developed as helmet bristles (Kristiansen, 1971). Fott (1962) distinguished four varieties of *M. acaroides* based on bristle characters, but the three of them, excluding the very deviating var. *inermis* Fott, should be united as *M. acaroides* var. *acaroides*, with a variable ratio between helmet bristles and serrated bristles (see Asmund & Kristiansen, 1986). It is not known whether bristle morphology in this species depends on temperature,

such as is the case in *M. crassisquama* (Asmund) Fott (see Siver & Skogstad, 1988). *M. acaroides* has previously been recorded from Bulgaria by LM by Valkanov (1928); the identification was later confirmed by EM (Kristiansen, 1971). Further LM identifications include records by Valkanov (1934), Vodenicharov (1962), Szemes (1967), Valchanova (1995) and Stoyneva & Valchanova (1997). It is widely distributed in the northern temperate and subarctic regions, and it has also been recorded from Romania and Hungary (Asmund & Kristiansen, 1986; Kiss & Kristiansen, 1994). *Mallomonas pumilio* var. *munda* (Fig. 18, only this single scale found) is distributed in temperate regions. It is widely recorded from Europe, and it has also been found in Canada and in temperate parts of S. America. In adjacent countries it has also been found in Romania (Momeu & Péterfi, 1987).

Only one species of the genus *Synura* has been found, viz. *Synura petersenii* (Fig. 19). It has also been found in the adjacent countries: Romania (Péterfi, 1966), Greece (Kristiansen, 1980), Hungary (Kristiansen & Padišák, 1992) and it is one of the world's most widely distributed chrysophyte. Another species has been recorded as frequent in Bulgaria, viz. *Synura uvella* Ehr. (Valkanov, 1926, 1928; Vodenicharov, 1962, 1967; Naidenov, 1964; Naidenov & Saiz, 1977; Saiz, 1978; Stoyneva, 1991; Valchanova, 1995), but without examination of the silica-scale structure. "*Synura uvella*" was previously used as a common name for any species of the genus, and thus it cannot be seen which species is actually referred to in these works (Kristiansen *et al.*, in press).

As regards the occurrence of the species in the examined localities, the following remarks can be made. There were no species reported from the acid peat bogs (localities 9, 10). The following species were confined to the mountain lakes (localities 12-21): *Spiniferomonas abei*, *Mallomonas matvienkoeae*, *M. papillosa*, *M. alpina*, and *M. pumilio* var. *munda*. This is in accordance with their general preference for somewhat oligotrophic conditions. *Paraphysomonas takahashii* and *P. vestita* were only found in the highly eutrophic lake loc. 2, as is often seen in this heterotrophic genus. The remaining species were found in a variety of locality types, reflecting that most of them, especially *Synura petersenii*, have a wide environmental range.

It is seen from the above that altogether 13 species were found during this investigation, only one of which has been recorded previously from Bulgaria by EM examinations, and two other from LM observations only. More species could certainly be found, and indeed several other species have been recorded from LM observations (Kristiansen *et al.*, in press). In the adjacent countries many more species of silica scaled chrysophytes have been found, e.g. in Greece 38 species of silica-scaled chrysophytes have been identified by EM (Kristiansen, 1980, 1983).

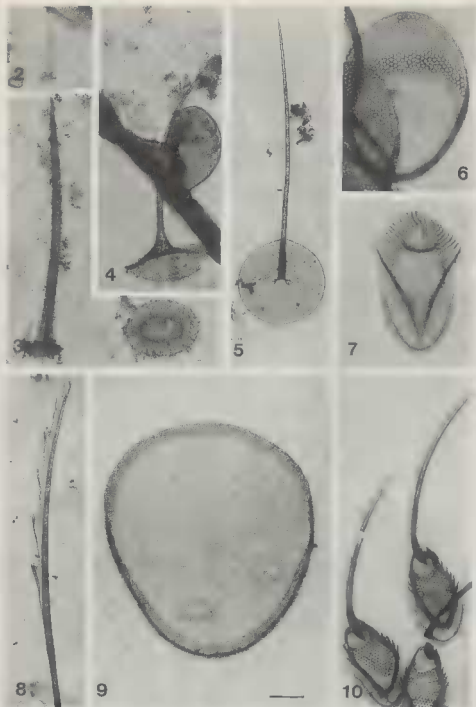
Most of the species are widely distributed and more or less cosmopolitan, except *Mallomonas acaroides*, *Mallomonas actinoloma* var. *maramuresensis* and *Paraphysomonas takahashii* which are restricted to the Northern Hemisphere, the two latter having very scattered occurrences. *Spiniferomonas abei* and *M. pumilio* var. *munda* have bipolar temperate occurrences (see Kristiansen & Vigna, 1996).

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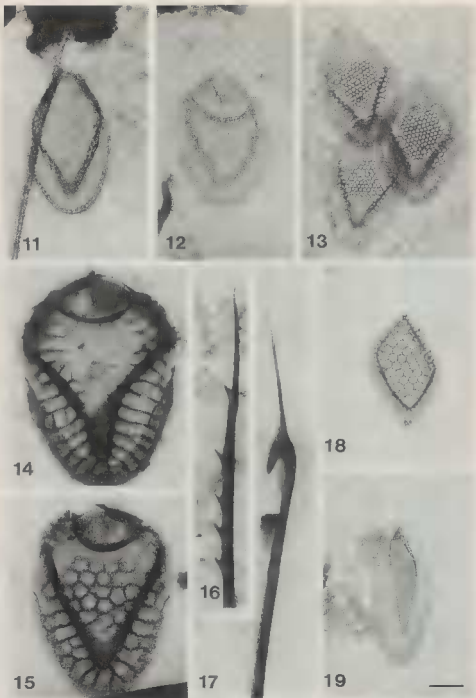
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Figs 2-10. Fig. 2. *Spiniferomonas abei*, spine scale (12). Fig. 3. *S. trioralis*, spine scale (2) and plate scale (19). Fig. 4. *Paraphysomonas takahashii*, spine scale (2). Fig. 5. *P. vestita*, spine scale (2). Fig. 6. *Mallomonas matvienkoei* f. *matvienkoei*, scale (12). Fig. 7. *M. actinoloma* var. *maramuresensis*, scale (19). Figs 8-9. *M. caudata*, bristle and scale (19). Fig. 10. *M. papillosa* f. *papillosa*, scales with bristles (21). Scale bar indicates 1 μ m, except in Fig. 8, 10 μ m. Locality numbers indicated.



Figs 11-19. Fig. 11. *Mullomonas alpina*, domeless body scale (19). Figs 12-13. *M. tonsurata*, apical and body scales (19 and 8). Figs 14-15. *M. acaroides* var. *acaroides*, body scales (16 and 18). Figs 16-17. *M. acaroides* var. *acaroides*, serrated bristle (18) and helmet bristle (20). Fig. 18. *M. pumilio* var. *munda*, body scale (20). Fig. 19. *Synura petersenii*, posterior body scale (19). Scale bar 1 μ m. Locality numbers indicated.